scientific reports

OPEN

Difference in nutritional status and food sources for hard- and soft-shell crabs (*Portunus trituberculatus*) using amino acids and isotopic tracers

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The swimming crab, *Portunus trituberculatus*, supports the world's largest crab fishery. Hard-shell crabs have fully developed exoskeletons and are rich in meat, while soft-shell crabs, which have recently molted, contain less meat. To determine their nutritional status and feeding behavior, we measured amino acids (AAs), organic carbon (OC), $\delta^{13}C$, $\delta^{15}N$, and ^{210}Po in the muscle of hard- and soft-shell crabs collected from the eastern Yellow Sea in May 2024. The concentrations of total AAs (TAAs) and OC were approximately 1.4 and 1.3 times higher, respectively, in hard-shell crabs than in soft-shell crabs. A significant positive correlation between TAAs and OC ($r^2 = 0.74$, $p < 0.05$) suggests that hard-shell crabs have a better nutritional status, due to the consumption of higher-quality food. Hard-shell crabs also exhibited significantly higher $\delta^{13}C$ values and ^{210}Po activities than soft-shell crabs, whereas $\delta^{15}N$ values showed no significant difference between the two groups. These results indicate that hard-shell crabs primarily consume higher trophic-level prey compared with soft-shell crabs, despite both occupying the same trophic position. Thus, this difference in food sources may be influenced by post-molt hiding behavior in soft-shell crabs, which limits access to high-trophic prey, or alternatively, by competition for food in coastal waters.

Keywords *Portunus trituberculatus*, Nutritional status, Food source, Amino acids, Stable isotopes, ^{210}Po

The swimming crab, *Portunus trituberculatus* (Miers, 1876), is widely geographically distributed in the Indian and West Pacific Oceans[1–3]. This species is primarily harvested for human consumption and is highly valued in seafood markets, especially in Southeast and East Asia, including Korea, China, Japan, and Taiwan[1–3]. The global catch of this crab has been steadily increasing since the 1970s, making it the largest crab fishery in the world, with over 540,000 tons being caught annually between 2013 and 2019[4].

In Korea, *P. trituberculatus* is one of the most important commercial crab species, with an annual catch of 15,000 tons between 2019 and 2023, showing a remarkable increase since 2018 (Korean Statistical Information Service; Fig. S1). Crab fishing occurs across all coastal waters of the Korean Peninsula, including the western, southern, and eastern coasts, with the Yellow Sea (the western coast) accounting for more than 80% of the total catch (Fig. S1). This species exhibits seasonal migration, and its spatial distribution varies significantly across the season[3]. In winter, crabs hibernate by burying themselves in muddy or sandy bottoms in deep areas (> 50 m depth)[3,5]. In spring, they migrate to shallow areas (about 5–30 m depth) in the Yellow Sea to spawn and grow during summer[3,5,6]. *P. trituberculatus* is an opportunistic omnivore, primarily feeding on benthic organisms such as bivalves, small crustaceans, polychaetes, zooplankton, as well as sediment organic matter (SOM)[7,8]. Its reproductive cycle is closely linked to seasonal migration[5,9]. Similar to many marine crabs, females are receptive to mating only immediately after molting, during the soft-shell stage. Mating typically occurs in late spring and early summer, coinciding with their migration to shallow waters. This species reproduces once or twice a year during summer, with a single reproductive event producing approximately 1 to 4 million eggs, which are laid

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after the shell has hardened[5,9]. In late autumn, crabs gradually migrate back to deeper waters for overwintering[3]. Thus, in Korea, crabs are primarily caught during spring and autumn, as fishing is restricted during the summer spawning season[10].

In general, adult crabs caught in the Yellow Sea have hard shells and a rich meat content[5]. However, in recent years, a significant portion of the catch has consisted of recently molted crabs, known as soft-shell crabs[10]. These crabs are usually caught in autumn when rapid physiological changes occur after spawning[10]. Due to their low meat content, physical weakness, and high susceptibility to stress, soft-shell crabs hold little commercial value and are invariably discarded by fishermen in autumn[10]. Their increasing abundance could negatively impact fishery yields and economic returns. Thus, soft-shell crabs pose a major issue for the crab fishery, as they constitute up to ~60% of the catch at times.

The crab naturally grows through the molting process during its lifetime. In portunid crabs, molting occurs at least fourteen times from the larval stage to juvenile stage before reaching maturity, after which adult crabs typically molt once or twice a year[11,12]. To molt, the crab rapidly absorbs water, splits its old shell, and backs out of it. The hardening of the new shell begins within 12 h of molting and continues for up to 30 days afterward[13,14]. Thus, the hardness for a crab's shell depends on the time elapsed after molting. The hardening time of the crab's shell, particularly, is influenced by external environmental conditions, such as water temperature, mineral availability, and food quality[15–19].

In this study, we measured amino acids (AAs), organic carbon (OC), stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), and radionuclide ^{210}Po in the muscle of hard-shell and soft-shell crabs from the eastern Yellow Sea in May 2024, to determine their nutritional status and feeding behavior associated with the occurrence of soft-shell and hard-shell crabs. In general, AAs in marine organisms can be used as an indicator of nutritional status[20,21], and OC represents energy storage. We also, for the first time, attempted to evaluate the differences in food sources between the two crab types by measuring $\delta^{13}C$, $\delta^{15}N$, and ^{210}Po in muscle, which are related to an organism's trophic level[22–24]. A better understanding of their feeding ecology will provide valuable insights into trophic dynamics, resource competition, and ecological shifts affecting crab populations. These insights may contribute to the development of fishery management strategies to address the occurrence of soft-shell crabs.

Results and discussion

Morphological characteristics of hard- and soft-shell crabs

Among the 57 crabs used in this study, 27 individuals (avg. 79 ± 7 DU) were classified as hard-shell crabs, while the remaining 30 individuals (avg. 54 ± 9 DU) were classified as soft-shell crabs (Fig. 1a). The results of the multivariate analysis of variance (MANOVA) indicated significant overall differences in morphological and biochemical parameters between hard-shell and soft-shell crabs (Pillai's trace = 0.786, $p < 0.05$; Table S1). Similarly, principal coordinates analysis (PCoA) revealed a clear biochemical separation between hard-shell and soft-shell crabs, primarily driven by OC, AAs, $\delta^{13}C$, and ^{210}Po (Fig. S2). The body weight (BW) of hard-shell crabs ranged from 215 to 317 g (avg. 266 ± 27 g), which showed no significant difference compared with that (182–336 g, avg. 251 ± 35 g) of soft-shell crabs ($p = 0.45$; Fig. 1b, Table S1). The carapace length (CL; 72–81 mm, avg. 78 ± 3 mm) and carapace width (CW; 122–139 mm, avg. 134 ± 5 mm) of hard-shell crabs also did not show significant differences compared with those (71–84 mm, avg. 77 ± 2 mm and 125–145 mm, avg. 132 ± 5 mm, respectively) of soft-shell crabs ($p = 0.11$ and 0.07, respectively; Fig. 1c, d, Table S1). In general, an adult crab, when grown for 12–20 months, reaches BW of about 234–359 g and CW of about 127–148 mm (https://www.nifs.go.kr). Thus, both hard-shell and soft-shell crabs used in this study seem to be in a similar lifecycle phase.

Nutritional status of hard- and soft-shell crabs based on amino acids

The concentrations of OC and total AAs (TAAs; the sum of individual AAs) in the muscle of hard-shell crabs ranged from 120 to 291 mg g^{-1} (avg. 180 ± 33 mg g^{-1}) and from 5 to 26 mg g^{-1} (avg. 15 ± 6 mg g^{-1}), respectively, which were approximately 1.3 and 1.4 times higher than those (81 – 174 mg g^{-1}, avg. 136 ± 21 mg g^{-1} and 6 – 17 mg g^{-1}, avg. 11 ± 2 mg g^{-1}, respectively) in soft-shell crabs ($p < 0.05$, Fig. 1e, 1f, Table S1). Generally, during the molting process, crabs rapidly absorb water, increasing muscle moisture, which is later replaced by protein as the shell hardens[25]. During this process, AAs are synthesized in the muscle to provide energy for ecdysis and removal of the shell[26]. Thus, our result suggests that hard-shell crabs synthesize larger amounts of AAs in their muscle relative to soft-shell crabs. This result is also supported by a significant positive correlation between OC and TAA concentrations ($r^2 = 0.74$, $p < 0.05$; Fig. 2a). Our result is consistent with previous studies, which reported that the concentrations of TAAs in the muscle of hard-shell crabs were significantly higher than those in soft-shell crabs[10,27,28].

In general, most animal species cannot synthesize EAAs on their own and therefore must obtain them from their food[29]. Thus, the concentration of essential AAs (EAAs) and the ratio of EAAs/TAAs in organisms are key factors for evaluating their nutritional status[20,21]. In this study, the concentrations of EAAs (1.2–7.7 mg g^{-1}, avg. 4.8 ± 1.7 mg g^{-1}) and ratios of EAAs/TAAs (0.14–0.58, avg. 0.33 ± 0.10) in the muscle of hard-shell crabs were approximately 2.7 and 1.9 times higher than those (0.6–3.4 mg g^{-1}, avg. 1.8 ± 0.9 mg g^{-1} and 0.06–0.51, avg. 0.17 ± 0.10, respectively) in soft-shell crabs, respectively ($p < 0.05$; Fig. 1g, 1h, Table S1). These results suggest that hard-shell crabs are in a better state of nutrition than soft-shell crabs, likely due to active feeding on food with high-quality protein.

Food sources of hard- and soft-shell crabs based on $\delta^{13}C$, $\delta^{15}N$, and ^{210}Po

The $\delta^{13}C$ values (−17.24–−14.41‰, avg. −16.14 ± 0.79‰) in the muscle of hard-shell crabs were significantly enriched relative to those (−18.77–−16.04‰, avg. −17.18 ± 0.72‰) in soft-shell crabs ($p < 0.05$; Fig. 3). However, there was no significant difference in $\delta^{15}N$ values between hard-shell (10.07–13.03‰, avg. 11.58 ± 0.71‰) and soft-shell crabs (9.46–13.76‰, avg. 11.37 ± 1.07‰) ($p = 0.40$; Fig. 3, Table S1). Based on the $\delta^{15}N$ values of

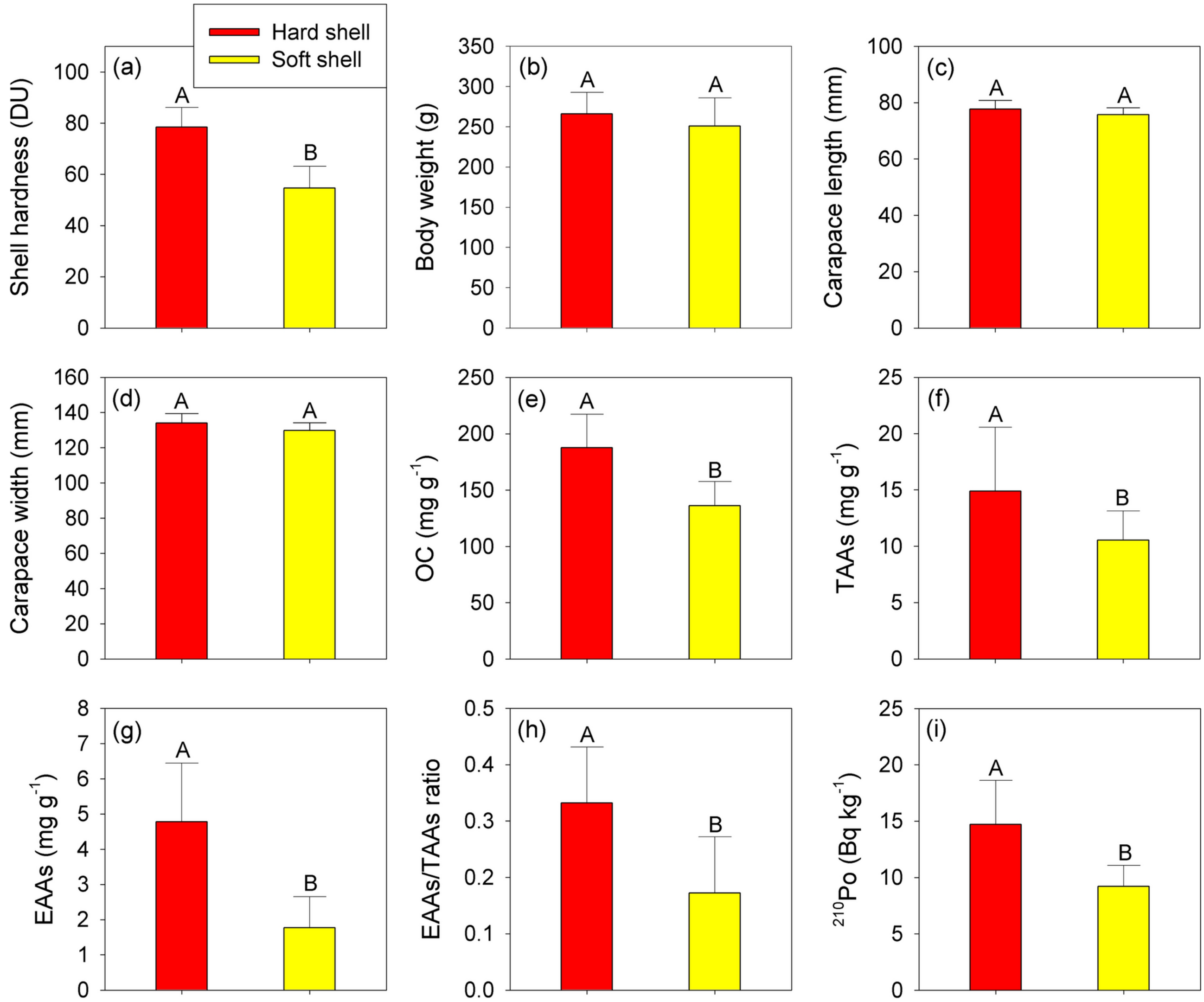

Fig. 1. Averages and standard deviations of (**a**–**d**) morphological and (**e**–**i**) biochemical parameters in hard-shell (n = 27) and soft-shell (n = 30) crabs from the eastern Yellow Sea. Different letters indicate significant differences ($p < 0.05$).

zooplankton (trophic level: 2) as primary consumers, previously measured in the eastern Yellow Sea[7], the trophic level of hard-shell crabs (avg. 2.76 ± 0.21) was similar to that of soft-shell crabs (avg. 2.70 ± 0.32). Feng et al.[30] suggested that species at the same trophic position do not necessarily have equal $\delta^{13}C$ values, as species at the same trophic level might utilize different food sources. Thus, differences in $\delta^{13}C$ and $\delta^{15}N$ values between hard-shell and soft-shell crabs suggest that they consume different types of food sources even though they occupy the same trophic level. Soft-shell crabs, in particular, may have reduced access to mobile, high trophic-level prey due to behavioral constraints after molting, contributing to their lower $\delta^{13}C$ values[31].

The average values of $\delta^{13}C$ and $\delta^{15}N$ in the five groups of potential food sources were significantly more depleted than those in both hard-shell and soft-shell crabs (Fig. 3). Among these groups, the trophic level of crustaceans (avg. 2.61 ± 0.21) was the highest, followed by that of polychaetes (avg. 2.44 ± 0.09) and bivalves (avg. 2.33 ± 0.17), which were relatively lower than those of both hard-shell and soft-shell crabs (Table S1). The results of IsoSource modeling indicated noticeable differences in major food sources between hard-shell and soft-shell crabs (Fig. 4, Table S2). Crustaceans were identified as the primary food source for hard-shell crabs, contributing approximately 68% of their food (Fig. 4, Table S2), consistent with the feeding patterns observed in other portunid crabs such as *P. segnis* and *P. pelagicus*[32,33]. On the other hand, bivalves and polychaetes were major food sources for soft-shell crabs, accounting for approximately 32% and 30% of the total food consumption, respectively (Fig. 4, Table S2). These results suggest that differences in food sources may be attributed to the hiding behavior of soft-shell crabs to avoid predation[31], which likely increases their reliance on sedentary or slow-moving organisms such as bivalves and polychaetes. Alternatively, competition for food between hard-shell and soft-shell crabs may influence their feeding behavior. As such, a significant positive correlation between $\delta^{13}C$ values and TAA concentrations indicates that hard-shell crabs synthesize more AAs by consuming higher trophic-level organisms ($r^2 = 0.60$, $p < 0.05$; Fig. 2b).

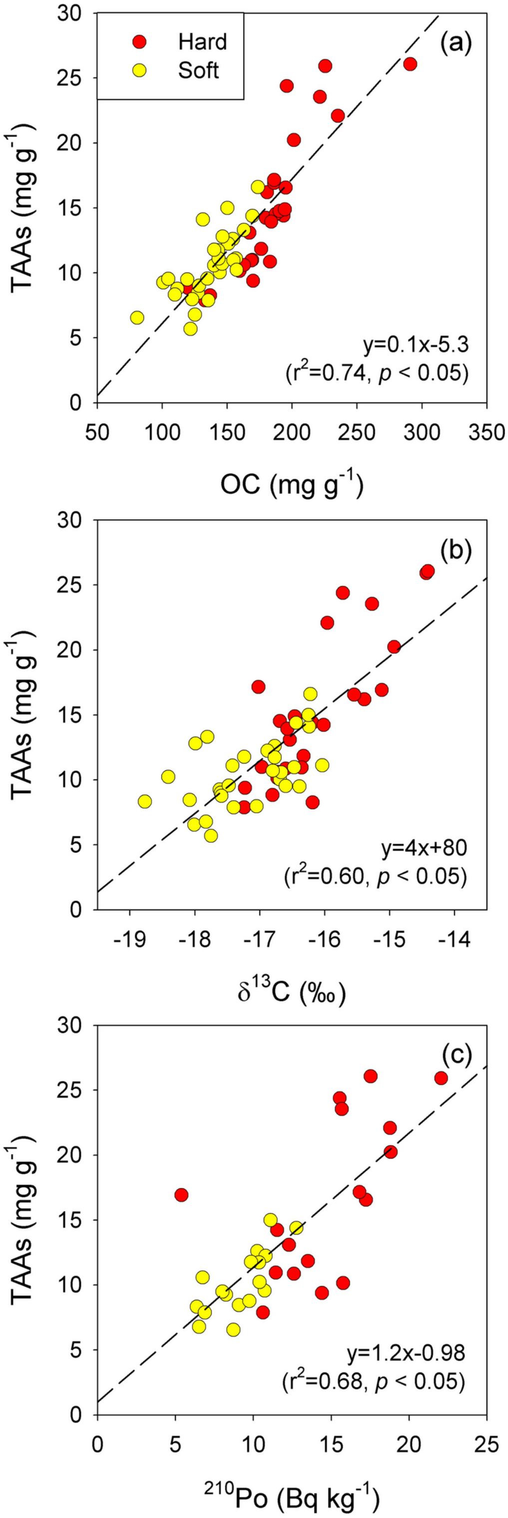

Fig. 2. Relationships between total amino acids (TAAs) and (**a**) organic carbon (OC), (**b**) $\delta^{13}C$, or (**c**) ^{210}Po in the muscle of hard-shell (n = 27) and soft-shell (n = 30) crabs from the Eastern Yellow Sea.

In general, ^{210}Po in marine organisms tends to accumulate at higher trophic levels as it moves along the food chain, since the main route of this radionuclide's accumulation in biota is through the ingestion of food[34–36]. Strady et al.[37] showed that ^{210}Po accumulates along the food webs from plankton to fish in the ecosystem of the Mediterranean Sea, France, as evidenced by a significant positive correlation between ^{210}Po and $\delta^{15}N$. In this study, the activities of ^{210}Po (5.4–22.1 Bq kg^{-1}, avg. 14.7 ± 3.9 Bq kg^{-1}) in the muscle of hard-shell crabs were approximately 1.6 times higher than those (6.4 – 12.8 Bq kg^{-1}, avg. 9.2 ± 1.9 Bq kg^{-1}) in soft-shell crabs

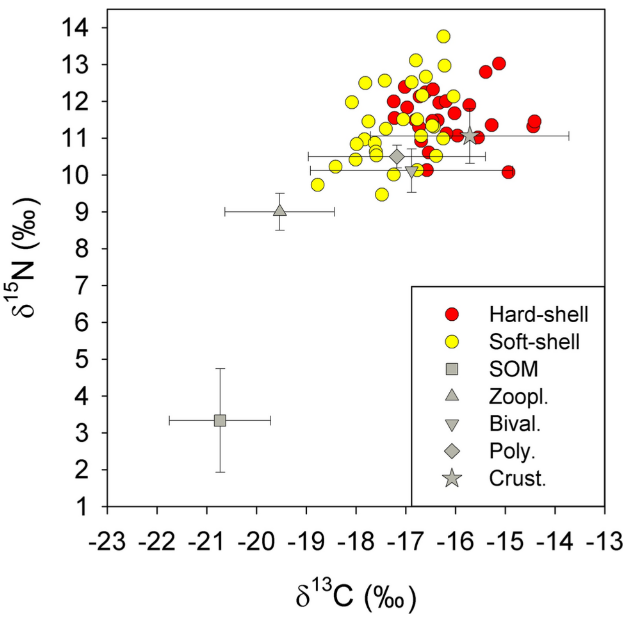

Fig. 3. Dual isotope plot of δ^{13}C and δ^{15}N for hard-shell (n = 27) and soft-shell (n = 30) crabs and their potential food sources in the eastern Yellow Sea. Data for δ^{13}C and δ^{15}N values of sediment organic matter (SOM), zooplankton (Zoopl.), bivalves (Bival.), polychaetes (Poly.), and crustaceans (Crust.) were obtained from NIFS (2023). δ^{13}C and δ^{15}N values for SOM, Zoopl., Bival., and Poly. are presented as average ± standard deviation.

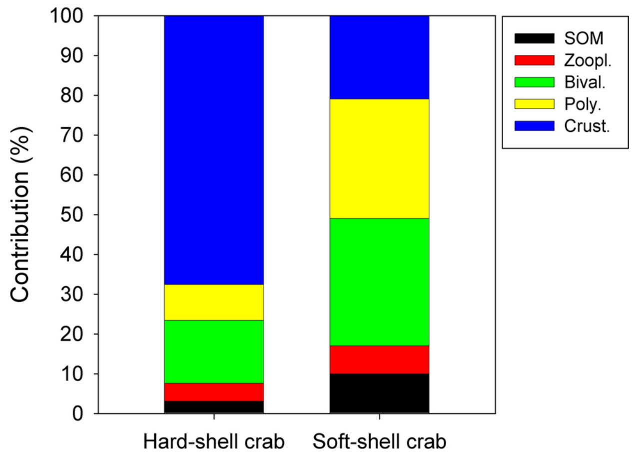

Fig. 4. Contributions of sediment organic matter (SOM), zooplankton (Zoopl.), bivalves (Bival.), polychaetes (Poly.), and crustaceans (Crust.) to the food of hard-shell and soft-shell crabs in the eastern Yellow Sea.

($p < 0.05$; Fig. 1i, Table S1). A significant positive correlation between ^{210}Po activities and TAA concentrations ($r^2 = 0.60$, $p < 0.05$; Fig. 2c) further supports that hard-shell crabs consume more organisms at high trophic level. Considering the fact that ^{210}Po has a relatively short half-life of about 138 days, its higher activities in hard-shell crabs suggest that these crabs are continuously exposed to ^{210}Po through their food. Thus, the sustained intake of ^{210}Po from food likely offsets the radioactive decay that would otherwise reduce its activity over time, leading to its accumulation in muscle tissue.

Recently, Park et al.[38] reported a rapid decline in nutrient concentrations in the coastal waters of the eastern Yellow Sea over the past three decades. This circumstance could potentially lead to a decrease in phytoplankton biomass, which may subsequently reduce the biomass of higher trophic organisms[39,40]. However, unusually in the eastern Yellow Sea, crab catches have continuously increased in spring over the last five years, perhaps due to environmental changes (i.e. temperature). This trend may intensify competition for food within crab populations. Similar patterns were observed in other marine crabs, such as *Callinectes sapidus* and *P. pelagicus*, where shifts in temperature, nutrients, and primary productivity affected population dynamics and heightened competition for food[41,42]. Thus, our results suggest that the occurrence of soft-shell crabs may be influenced by a greater proportion of individuals experiencing delayed exoskeleton hardening, likely due to limited access to high-quality food caused by heightened competition under limited primary production in the eastern Yellow Sea. Since this study was conducted only in spring and did not fully account for environmental conditions influencing shell hardening, further extensive studies are necessary to examine seasonal and long-term changes in environmental conditions, including temperature, salinity, nutrients, and primary production, and their effects on crab production and the occurrence of soft-shell crabs.

Conclusions

Our results suggest that the nutritional status and feeding behaviors of hard-shell and soft-shell crabs are influenced by ecological and physiological constraints. The higher concentrations of organic carbon (OC), total amino acids (TAAs), and essential amino acids (EAAs) in hard-shell crabs indicate more active amino acid synthesis and overall better nutritional status. The significant positive correlation between TAAs and OC suggests that protein synthesis is more active in hard-shell crabs, likely due to their active consumption of high-quality protein sources. Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and ^{210}Po activities further suggest that hard-shell crabs primarily consume prey at a higher trophic level than soft-shell crabs, although both groups occupy the same trophic position within the ecosystem. This dietary difference may be influenced by post-molt behavior in soft-shell crabs, which reduces opportunities to feed on high-trophic prey. Alternatively, heightened competition for food under limited primary productivity in the eastern Yellow Sea may contribute to their dietary patterns. In conclusion, our findings provide valuable insights into the ecological interactions and physiological constraints influencing the feeding ecology of crabs in dynamic coastal environments.

Materials and methods

Sample preparation

Non-ovigerous female crab samples (n = 57) were randomly caught by fishermen from the coastal waters off Incheon, located in the eastern Yellow Sea, in May 2024 (Fig. S1). The crab samples were stored at -20℃ and dissected within one day of purchase. The external morphology of the crab samples was determined by measuring body weight, carapace length, and carapace width. In this study, the hard-shell and soft-shell crabs were classified based on shell hardness measured using a digital durometer[10,43,44]. We defined crabs with carapace hardness ≥ 70 durometer unit (DU) as hard shell and those with hardness < 70 DU as soft shell[10,43,44]. After the morphological measurements, the entire claw musculature was dissected using scissors and forceps and small sub-samples were taken for biochemical and isotopic analysis. The extracted muscle samples were then freeze-dried, homogenized, and stored at -20℃ until analysis.

Analytical method

AA compositions were determined after acid hydrolysis. Approximately 0.1 g of muscle samples were hydrolyzed for 24 h at 110℃ with 12 M HCl in sealed glass tubes filled with N_2. The hydrolyzed samples were then evaporated to dryness using a freeze dryer to remove residual HCl and were dissolved in 10 mL of Milli-Q water. The hydrolysates were derivatized with *o*-phthaldialdehyde and measured using an ACQUITY ultra performance liquid chromatography (UPLC) system equipped with an AccQ-Tag Ultra C18 column (2.1 × 100 mm, 1.7 μm particles). A linear gradient of 25 mM sodium acetate (pH 6) and acetonitrile was used as described by Lee et al.[45]. The retention times and peak areas of the amino acid standard (AAS18, Sigma-Aldrich, USA) were compared to identify and quantify the individual AAs. AA data were corrected for reagent and procedural blanks, which were < 5% of the average sample concentrations. In this study, fourteen AAs were detected, including aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), glycine (Gly), arginine (Arg), alanine (Ala), tyrosine (Tyr), valine (Val), lysine (Lys), isoleucine (Ile), phenylalanine (Phe), leucine (Leu), and histidine (His). Among these individual AAs, EAAs include Thr, Val, Ile, Leu, Lys, Arg, His, and Phe.

The muscle samples for OC analysis were fumigated with concentrated HCl (37%) in a desiccator to remove inorganic carbon ($CaCO_3$) without rinsing the samples to minimize OC loss, and then dried at 50℃ for 12 h[46]. Approximately 1 mg of the samples was wrapped in a tin capsule, and measured using an elemental analyzer (EA 2400 Series II, PerkinElmer, USA). The uncertainties for the OC data were within 5%.

Prior to $\delta^{13}C$ analysis, the muscle samples were treated with concentrated HCl (37%) without rinsing to remove $CaCO_3$, and then dried at 50 °C, as $CaCO_3$ can elevate $\delta^{13}C$ values higher than those of organic animal tissues[46,47]. No acid treatment was applied for $\delta^{15}N$ analysis to prevent potential $\delta^{15}N$ enrichment[48]. Lipids were not extracted from the muscle samples, as *P. trituberculatus* muscle has a very low lipid content, and previous study has shown that lipid extraction does not significantly affect $\delta^{13}C$ values in crustacean tissues[49]. After pretreatment, approximately 1 mg of the samples was wrapped in a tin capsule and analyzed using an elemental analyzer (EA 3000, Eurovector, Italy) coupled to an isotope ratio mass spectrometer (Isoprime, Elementar, Germany). Stable isotope ratios are expressed in standard δ-unit notation, as follows:

$$\delta^{13}\mathrm{C}\ \text{or}\ \delta^{15}\mathrm{N}(\text{‰}) = \left[\frac{\mathrm{R_{sample}}}{\mathrm{R_{standard}}} - 1\right] \times 1000$$

where $R_{standard}$ and R_{sample} represent the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios in the standard and the sample, respectively. The accuracy was validated using certified reference material ($\delta^{13}C$: -26.39 ± 0.04‰ and $\delta^{15}N$: −4.52 ± 0.1‰; USGS40), and the uncertainties of the reference material were < 5%.

For ^{210}Po analysis, we randomly selected a total of 34 individuals from 57 crabs, comprising 17 hard-shell crabs and 17 soft-shell crabs. Approximately 1 g of the muscle sample was digested in concentrated HNO_3 and H_2O_2 for 24 h with an internal ^{209}Po spike (1.5 dpm). The solution was evaporated to dryness to remove HNO_3. The resulting residue was dissolved in 0.5 M HCl and heated on a hot plate at 80℃ for 1 h after adding ascorbic acid for iron reduction. The ^{210}Po in this solution was then spontaneously plated onto a silver disc while rotating the disk for 3 h using a magnetic stirrer. The ^{210}Po plated onto the silver disk counted for α activity using an alpha spectrometry with a passivated implanted planar silicon detector (Alpha Analyst, Canberra, Australia).

Food source contributions for hard-shell and soft-shell crabs

In this study, we evaluated the contributions of food sources for hard-shell and soft-shell crabs using the IsoSource mixing model[50]. This model is a mass balance mixing model that iteratively calculates all possible combinations of source contributions (0–100%) in small increments (typically 1%) to match the observed mixture's isotopic signatures within a defined tolerance level (e.g., 0.1–0.5‰). For this estimation, the potential food sources considered were SOM, zooplankton, bivalves, polychaetes, and crustaceans, based on the feeding ecology of *P. trituberculatus*[7,8]. The δ^{13}C and δ^{15}N values for these sources were obtained from previous measurements conducted in the eastern Yellow Sea (Table S3)[7], from regions similar to the crab fishing area and during the crabs' growth season. The source increment was set at 1%, and the initial tolerance was set at 0.1‰, increasing up to 0.5‰ if necessary. The δ^{13}C and δ^{15}N values were used after correcting for trophic fractionation effects of 1‰ for δ^{13}C and 3.4‰ for δ^{15}N[24,47,51]. The contributions of each source were estimated based on their average values, as well as their minimum and maximum feasibility.

Statistical analysis

Data are presented as average ± standard deviation (AVG ± SD). MANOVA was conducted using SPSS 26 (SPSS Inc., Illinois, USA) to assess overall differences in morphological (BW, CL, and CW) and biochemical (OC, TAAs, EAAs, δ^{13}C, δ^{15}N, and ^{210}Po) parameters between hard-shell and soft-shell crabs. Prior to analysis, homogeneity of covariance matrices was tested using Box's M test, and homogeneity of variances for each dependent variable was assessed with Levene's test. Data normality was evaluated using the Shapiro–Wilk test. Pillai's trace test was used to evaluate the multivariate effect, with significance set at $p < 0.05$ (95% confidence level). Subsequently, multiple independent ANOVA tests were conducted to evaluate differences in individual parameters between the two groups. PCoA with Euclidean distance was performed using the vegan package in R (v4.4.3, R Core Team) to visualize differences in morphological and biochemical parameters between the two groups. Additionally, simple linear regression analyses were conducted to examine the relationships between TAAs and OC, δ^{13}C, and ^{210}Po, to evaluate the link between the crabs' nutritional status and the trophic levels of their food sources. The assumptions of normality and homogeneity of variances were tested using the Shapiro–Wilk and Levene's tests, respectively. Since all assumptions were met, simple linear regression analysis was applied with significance set at $p < 0.05$.

Data availability

The data generated in this study are provided in the Supplementary Information.

Received: 26 October 2024; Accepted: 22 April 2025
Published online: 05 May 2025

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Acknowledgements

We would like to thank the members of the Environmental and Marine Biogeochemistry Laboratory (EMBL) for their assistance with the laboratory analyses. This study was supported by a grant from the National Institute of Fisheries Science, Korea (R2025010) and by the National Research Foundation of Korea (NRF) grant funded by the Korea government (RS-2023-NR076495).

Author contributions

G.K. conceived and designed this study. H.K.K and G.K analyzed the data and wrote the manuscript. H.K.K and N.K performed chemical analyses. Y.K.C, J.H, Y.C, W.A.L, S.J.L, and J.B.L contributed to the interpretation of the results. All authors discussed the results and commented on the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-99629-x.

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